Marking Tephritidae Fruit Fly Adults in Hawaii for Release-Recovery Studies[1,2,3,4]

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The need to identify released insects is paramount in studies based on release-recovery evaluations. In Hawaii identification of released oriental fruit flies, *Dacus dorsalis* Hendel, is accomplished by rearing and releasing phenotypically distinct flies (Steiner *et al.*, 1962). Presently, marking the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), and the melon fly, *D. cucurbitae* Coquillett, is accomplished with the use of Calco Blue® dye (Steiner, 1965), Blaze Orange Day-Glo® fluorescent dye (Holbrook *et al.*, 1970) or Tinopal® SFG fluorescent whitening agent (Schroeder *et al.*, 1972). Because of the need for additional markers, we routinely examine dye materials as possible external (ptilinum dye) and internal (vital dye) markers. These continuing studies include coating pupae with dye powder to determine the potential of the material as an external adult dye and addition of the dye to the larval diet followed by examination of the adult to determine the potential of the material as a vital dye. Promising materials are then evaluated for retention by the insect and ease of detection, and dyed insects are examined for behavioral changes. Finally the material is evaluated in a field release-recovery test. This report describes the general procedure used in dyeing and examining adult flies for the marker, lists materials tested, and discusses dyes presently used in Hawaii.

General.—Adult Tephritidae are marked with an external dye by tumbling a known quantity of pupae with a known quantity of dry powder. Dye adhering to the pupae and dye on surrounding pupae is contacted by the ptilinum at adult eclosion and the material is retracted into the head of the fly along with this structure. Dye particles that adhere to other body parts are usually disposed of by the continual "cleaning" activity of the adult. Therefore, when examining for the dye marker the head of the fly must be crushed. A blunt object such as a flattened bolt tip is used to crush heads of flies placed on filter paper. The bolt is dipped in acetone before being pressed against the head and the acetone washes the dye out onto the filter (Steiner, 1965). Persons crushing heads soon develop the technique so as to get sufficient acetone on the head but not too much so that the spread dye spot is over-diluted and faint. When Calco Blue dye is used as the marker, the head must be dry when examined to avoid a masking of the dye by head fluids and retinal pigments. The same procedure is followed

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when examining for blaze orange or Tinopal except that the filter paper is examined under UV light. Also when Tinopal is used, flies can be dropped in a petri dish containing acetone that is illuminated with UV light: Tinopal will glow bright blue.

Methods and Materials.—*Preliminary testing of candidate external dyes.*—The melon fly is used as the test species and 0.5-2.0g of undiluted dye powder mixed with ca. 100 ml of pupae (35,000/liter). Pupae are then examined for dye retention and if the material adheres to the pupae, adults emerging from the 100-ml mass of pupae are examined for the dye as described.

Preliminary examination for vital dyes.—Candidate internal dye markers are dissolved in acetone, vegetable oil or water depending on the solubility of the dye, then incorporated into the standard larval medium (Tanaka *et al.*, 1969) at a concentration of 1.0, 1.5, and 2.0 g of dye/liter of diet. Pupae are held in vermiculite and emerging adults examined for the presence of an internal color. If a color is present, flies are retained and additional examination made with increasing adult age. All 3 species of Tephritidae found in Hawaii are tested using the specific artificial diet with the incorporated dye material.

Results and Discussion.—Among the 51 materials evaluated we found 3 groups of dye materials that were suitable as external markers and 1 material, Sudan® Deep Black BB, that acted as a vital dye when added to the melon fly larval diet. Materials were classified as follows: A—Acceptable, presently used to mark fruit flies in Hawaii. B—Potentially useful but with the following limitations: 1. Resembles body pigments. 2. Detection difficult usually because the material could not be washed from head capsule. 3. Material water soluble and difficult to handle because of excessive staining. 4. Poor adhesion, due to coarse texture. C—No potential as a marker (Table 1).

External dyes.—The Calco oil dyes have been used extensively as external markers in major field releases; however, Calco Oil Blue, the preferred material, is no longer being manufactured. Calco Red and Calco Orange have also been used in release-recovery work but are inferior to Calco Blue.

The Day-Clo dyes are suitable markers, with Blaze Orange the preferred material. The other colors, although acceptable, closely resemble each other, are more difficult to detect, or can be mistaken for natural body pigments.

Tinopal, which has been evaluated in field studies, is easy to detect, both by crushing heads or by immersing flies in acetone, and is an acceptable external marker.

Internal dye.—Sudan Deep Black BB colors the adult melon fly deep black when 1 g of dye material dissolved in a small quantity of vegetable oil is added to 1 liter of larval diet. The dye becomes incorporated in the hemolymph of the larva and adult. The color is slowly eliminated from the adult hemolymph in ca. 2 weeks,; however, the 4 rectal papillae are permanently dyed deep blue. In field evaluations the material was difficult to detect due to the need for internal examination of the trapped insects. For

this reason and because of changes in behavior of the dyed adults (Schroeder in press) use of the material has been limited to laboratory studies.

TABLE 1. *Dye materials evaluated as possible external and internal adult fly markers.*

Source	Dye	Results External	Internal
Du Pont	Fuchsine N (Colour Index 42520, Basic Violet 2)	C	C
	Anthraquinone Green GNN (CI 6157 Acid Green 25)	C	C
	Pontacyl Blue Black Sx (CI 20470 Acid Black 1)	C	C
BASF Wyandotte	Sudan ® Blue 11	B-4	C
	Sudan Orange R	C	C
	Sudan Yellow 3G	C	C
	Sudan Red 7B	C	C
	Sudan Deep Black BB	C	B-2
Nutritional Biochemicals Corp.	Fluorescent Probe, 1 Anilino-8-Naphthalene Sulfonic Acid	C	C
	Flazo Orange	C	C
	Superchrome Blue B Extra	C	C
	Diamond Black	C	C
	Diamond Red	C	C
	Eriochrome Black	C	C
	Ponta Chrome Blue Black ZF	C	C
	Pontachrome Violet SW	C	C
	Acid Alizarin	C	C
Eastman Kodak Co.	Methyl-5, 12-diethylfluoridinum methosulfate, Eastman organic number 11258	C	C
General Aniline and Film Corp.	Rhodamine B Extra S	B-3	C
	Genacryl Yellow 4G	C	C
	Genacryl Red 4B	C	C
	Genacryl Pink 3G	C	C
	Azosol Brilliant Yellow 6GF	C	C
Hartman Leddon Co.	Brilliant Yellow	C	C
	Brilliant Green	C	C
	Bismarck Brown Y	C	C
Switzer Bros.	Day-Glo ® Daylight Fluorescent Pigment Blaze Orange A15	A	C
	Day-Glo Neon Red A12	A	C
	Day-Glo Aurora Pink A11	A	C
	Day-Glo Saturn Yellow A17	B-1	C
	Day-Glo Signal Green A18	A	C
	Day-Glo Horizon Blue A19	B-1	C
	Day-Glo Rocket Red A13	A	C
Ultra-Violet Products Inc.	Tracer Powder C205 Yellow	C	C
	Tracer Powder C204 Orange	C	C

TABLE 1. *Dye materials evaluated as possible external and internal adult fly markers.*

Source	Dye	Results External	Results Internal
Geigy Industrial Chem.	Tinopal ®BHS	C	C
	Tinopal GS	C	C
	Tinopal PCR	C	C
	Tinopal SFG	A	C
American Cyanamid	Calco ®Oil Red N1700	A	C
	Calco Oil Blue	A	C
	Calco Oil Orange Y293	B-4	C
	Calco Oil Ornage 7078-V	B-4	C
The Blue Line Chemical Co.	Dophenco Gelatin Capsules	C	C
U.S. Radium Corp.	Helecon 1757	B-2	C
	Helecon 1953	B-2	C
	Helecon 2267	B-2	C
	Helecon Phosphorescent 2304	C	C
	Helecon Phosphorescent 2315	C	C
	Helecon Phosphorescent 2330	C	C
	Helecon Phosphorescent 2478	C	C

A—Acceptable, presently used to mark fruit flies in Hawaii.
B—Potentially useful but with the following limitations:
1. Resembles body pigments.
2. Detection difficult usually because the material could not be washed from the head capsule.
3. Material water soluble and difficult to handle because of excessive staining.
4. Poor adhesion due to coarse texture.

C—No potential as a marker.

REFERENCES CITED

Holbrook, F. R., L. F. Steiner and M. S. Fujimoto. 1970. Mating competitiveness of Mediterranean fruit flies marked with fluorescent powders. J. Econ. Entomol. 63:454-455.

Schroeder, W. J., R. T. Cunningham, R. Y. Miyabara and G. J. Farias. 1972. A fluorescent compound for marking tephritidae. Ibid. 65:1217-1218

Steiner, L. F. 1965. A rapid method for identifying dye-marked fruit flies. Ibid. 58:374-375.

Steiner, L. F., W. C. Mitchell and A. H. Baumhover. 1962. Progress of fruit fly control by irradiation sterilization in Hawaii and the Mariana Islands. Int. J. Appl. Radiat. Isotop. 13:427-434.

Tanaka, N., L. F. Steiner, K. Ohinata and R. Okamoto. 1969. Low-cost larval rearing medium for mass production of oriental and Mediterranean fruit flies. J. Econ. Entomol. 62:967-968.